

Anderson et al.
Serial No.: 09/921,004
Page 2 of 12

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions and listing of the claims in the application:

LISTING OF THE CLAIMS:

Claim 1: (amended) A method of detecting at least one ~~low-molecular-weight~~ protein and/or peptide component in a biological body fluid comprising:

- a) fractionating proteins or peptides in said biological body fluid by molecular weight to produce a fractionated protein or peptide sample;
- b) separating a first fraction from said fractionated protein or peptide sample, said first fraction having substantially all proteins or peptides recoverable from the body fluid with a molecular weight greater than about 3 kDa and below about 30,000 daltons ~~filtration limits of a normal kidney found in the biological fluid~~;
- c) recovering said first fraction having substantially all the proteins or peptides with a molecular weight greater than about 3kDa and below about 30,000 daltons ~~the filtration limits of a normal kidney found in the biological fluid~~, and
- d) determining the proteins or peptides present in said first fraction.

Claim 2: canceled

Claim 3: (amended) The method of claim 1, wherein said biological body fluid is selected from the group consisting of urine, blood, tissue cytosol or other tissue fluid, cerebral spinal fluid, sputum, feces and sweat.

Claim 4: (amended) The method of claim 1, wherein said biological body fluid is urine.

Claim 5: (amended) The method of claim 1, wherein said fractionating step comprises separation of ~~low-molecular-weight protein or peptide~~ constituents by size exclusion chromatography.

Anderson et al.
Serial No.: 09/921,004
Page 3 of 12

Claim 6: (original) The method of claim 5, wherein said separation comprises sequential chromatography by separate stationary phases comprising different mesh sizes.

Claim 7: (amended) The method of claim 1, wherein said concentrating step comprises addition of further comprising adding at least one protease inhibitor to the body fluid upon collection.

Claim 8: (previously presented) The method of claim 1, wherein said fractionating step comprises a hydrodynamic step.

Claim 9: (original) The method of claim 8, wherein said hydrodynamic step is centrifugation.

Claim 10: (previously presented) The method of claim 1, further comprising fractionating said first fraction by elution from a reverse phase stationary phase.

Claim 11: (original) The method of claim 10 wherein said reverse phase is a non-porous C18 material.

Claim 12: (previously presented) The method of claim 1, wherein said first fraction is further fractionated by elution from an affinity column.

Claim 13: (previously presented) The method of claim 12, wherein said affinity column comprises monoclonal, polyclonal, recombinant, microorganism display antibodies, or fragments thereof.

Claim 14: (previously presented) The method of claim 13, wherein said monoclonal and/or polyclonal antibodies are directed to target proteins selected from the group consisting of albumin, transferrin, α_1 antitrypsin, α_2 macroglobulin, α_1 acid glycoprotein, C3,

Anderson et al.
Serial No.: 09/921,004
Page 4 of 12

Tamm-Horsfall protein, hemopexin, α_2 HS glycoprotein, α_1 antichymotrypsin, Gc globulin and ceruloplasmin.

Claim 15: (previously presented) The method of claim 13, wherein said affinity column is a non-immunologic entity comprising matrix.

Claim 16: (previously presented) The method of claim 15, wherein said non-immunologic entity is selected from the group consisting of protein A, protein G, haptoglobin, arginine, benzamidine, glutathione, Cibachron blue, calmodulin, gelatin, heparin, lysine, lectins, Procion Red HE-3B, nucleic acids and metal affinity media.

Claim 17: (previously presented) The method of claim 1, wherein said first fraction is further fractionated by electrophoresis.

Claim 18: (previously presented) The method of claim 1, wherein said first fraction is further fractionated by zonal sedimentation centrifugation on density gradients.

Claim 19: (previously presented) The method of claim 1, wherein said determining step comprises identifying said proteins or peptides by mass spectrometry or liquid chromatography.

Claims 20-24. (Canceled)

Claim 25: (previously presented) The method of claim 1 wherein said first fraction comprises native proteins.

Claim 26: (canceled)

Claim 27: (amended) The method of claim 1 further comprising recovering a second fraction from said biological body fluid having substantially all proteins with a molecular

Anderson et al.
Serial No.: 09/921,004
Page 5 of 12

weight above about 30,000 daltons and below about 75,000 daltons said filtration limits of a normal kidney found in said biological fluid and determining the proteins in said second fraction.

Claim 28: (canceled)

Claim 29: (previously presented) The method of claim 12 wherein said affinity column contains plural specific binding agents that bind to plural specific predetermined proteins.

Claim 30: (amended) The method of claim 1 wherein the biological body fluid is plasma or serum.

Claim 31: (amended) The method of claim 1 wherein said first and second fractions having substantially all the proteins or peptides recoverable from the body fluid with a molecular weight greater than about 3kDa and below about 75,000 daltons the filtration limits of a normal kidney found in said biological fluid consists essentially of plasma proteins or peptides capable of being filtered by a normal kidney.

Claim 32: (amended) The first fraction of a biological body fluid sample produced by the process of claim 1 wherein said first fraction having the proteins or peptides recoverable from the body fluid with a molecular weight greater than about 3kDa and below about 30,000 daltons the filtration limits of a normal kidney consists essentially of essentially all plasma proteins or peptides capable of being filtered by a normal kidney found in said biological body fluid within that molecular weight range.

Claim 33: (amended) The fraction of claim 32 wherein the biological body sample is urine.

Claim 34: (amended) The fraction of claim 32 wherein the biological body sample is plasma or serum.

Anderson et al.
Serial No.: 09/921,004
Page 6 of 12

Claim 35: (amended) The fraction of claim 32 wherein the biological body sample is from a tissue.

Claim 36: (canceled)

Claim 37: (previously presented) The method of claim 1 further comprising generating an antibody against at least one of said proteins or peptides.

Claim 38: (amended) The method of claim 37, further comprising:
contacting a test biological body fluid with said antibody against at least one of said proteins or peptides, and
detecting the presence or absence of said antibody binding to said protein or peptide.

Claim 39. (new) The method of claim 27, wherein said second fraction is further fractionated by elution from an affinity matrix specific for at least two specific proteins.

Claim 40. (new) The method of claim 39 wherein the two specific proteins are albumin and α_1 -acid glycoprotein.

Claim 41. (new) The second fraction of a body fluid sample produced by the process of claim 27 wherein said second fraction having proteins or peptides recoverable from the body fluid with a molecular weight greater than about 30,000 daltons and below about 75,000 daltons consists essentially of essentially all plasma proteins or peptides capable of being filtered by a normal kidney found in said body fluid.